Express Mail No. EL 501 636 477 US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Coruzzi et al.

Serial No.: To be assigned; Divisional of Serial No. 08/899,330 filed July 23, 1997

Group Art Unit: To be assigned

Examiner: To be assigned

Filed: On even date herewith

Attorney Docket No.: 5914-089

For: PLANT NITROGEN REGULATORY P-PII GENES

PRELIMINARY AMENDMENT UNDER 37 C.F.R. § 1.121

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Pursuant to 37 C.F.R. § 1.121, please enter the following amendments and remarks in connection with the above-identified application.

IN THE CLAIMS:

Cancel claims 1-6 and 9-10 without prejudice.

Add new claims as follows:

11. (New) A substantially pure nucleic acid molecule that hybridizes to the complement of a nucleotide sequence under highly stringent conditions, wherein said nucleotide sequence comprises a nucleotide sequence that encodes a P-PII gene product comprising:

(a) the amino acid sequence of SEQ ID NO:1; or

(b) the amino acid sequence of SEQ ID NO:2;

and wherein said substantially pure nucleic acid molecule encodes a functionally equivalent P-PII gene product.

12. (New) A substantially pure nucleic acid molecule that hybridizes to the complement of a nucleotide sequence under highly stringent conditions, wherein said nucleotide sequence comprises a nucleotide sequence that encodes a P-PII gene product comprising:

(a) the nucleotide sequence of SEQ ID NO:13;

(b) the nucleotide sequence of SEQ ID NO:14;

(c) the nucleotide sequence of SEQ ID NO:15; or

(d) the nucleotide sequence of SEQ ID NO:16;

and wherein said substantially pure nucleic acid molecule encodes a functionally equivalent P-PII gene product.

13. (New) A substantially pure nucleic acid molecule that hybridizes to the complement of a nucleotide sequence under moderately stringent conditions, wherein said nucleotide sequence comprises a nucleotide sequence that encodes a P-PII gene product comprising:

(a) the amino acid sequence of SEQ ID NO:1; or

(b) the amino acid sequence of SEQ ID NO:2.

14. (New) A substantially pure nucleic acid molecule that hybridizes to the complement of a nucleotide sequence under moderately stringent conditions, wherein said nucleotide sequence comprises a nucleotide sequence that encodes a P-PII gene product comprising:

(a) the nucleotide sequence of SEQ ID NO:13;

(b) the nucleotide sequence of SEQ ID NO:14;

(c) the nucleotide sequence of SEQ ID NO:15; or

(d) the nucleotide sequence of SEQ ID NO:16.

15. (New) The substantially pure nucleic acid molecule as in any one of claims 13-14, wherein said substantially pure nucleic acid molecule encodes a functionally equivalent P-PII gene product.

16. (New) A transgenic plant containing a transgene encoding a gene of interest operatively associated with a P-PII promoter, so that the gene of interest is expressed in shoots, roots or flowers of said transgenic plant.

17. (New) The transgenic plant of claim 16, wherein the gene of interest encodes a gene product that confers enhanced nitrogen utilization.

18. (New) The transgenic plant of claim 16, wherein the gene of interest encodes a gene product that confers herbicide resistance.

REMARKS

The subject application is a continuation application of application Serial No. 08/899,330 filed July 23, 1997. Claims 1-6 and 9-10 have been canceled without prejudice. New claims 11-18 have been added to more particularly point out and distinctly claim the present invention. Upon entry of the instant amendment, claims 7-8 and 11-18 will be pending. A courtesy copy of the pending claims is attached hereto as Exhibit A.

New claims 11-18 have been added. The new claims more specifically describe the subject matter that Applicants regard as the invention. Support for the new claims exists throughout the specification. Specifically, support for claims 11-18 can be found in the specification at, for example, page 13, line 5 to page 14, line 19. Support for claims 16-18 can be found in the specification at, for example, page 21, lines 15-28, and page 30, line 29 to page 33, line 17.

No new matter has been added. Entry of the foregoing amendments and remarks is respectfully requested.

CONCLUSION

Applicants respectfully request that the amendments and remarks made herein be entered and made of record in the file history of the present application.

No fee is believed to be due for this amendment. Should any fee be required, please charge the required fee to Pennie and Edmonds LLP Deposit Account No. 16-1150.

Respectfully submitted,

Date January 8, 2001

Laura A. Coruzzi — 30,742 (Reg. No.)

PENNIE & EDMONDS LLP
1155 Avenue of the Americas
New York, New York 10036-2711
(212) 790-9090

Enclosure

PLANT NITROGEN REGULATORY P-PII GENES

This invention was made with U.S. government support under National Institute of Health grant number GM32877 and Department of Energy grant number DE-FG02-94ER20133 and National Science Foundation grant No. DIR-8908095. The U.S. government has certain rights in the invention.

1. INTRODUCTION

The present invention generally relates to plant nitrogen regulatory PII gene (hereinafter P-PII gene), a gene involved in regulating plant nitrogen metabolism. The invention provides P-PII nucleotide sequences, expression constructs comprising said nucleotide sequences, and host cells and plants having said constructs and, optionally expressing the P-PII gene from said constructs. The invention also provides substantially pure P-PII proteins.

The P-PII nucleotide sequences and constructs of the invention may be used to engineer organisms to overexpress wild-type or mutant P-PII regulatory protein. Engineered plants that overexpress or underexpress P-PII regulatory protein may have increased nitrogen assimilation capacity. Engineered organisms may be used to produce P-PII proteins which, in turn, can be used for a variety of purposes including *in vitro* screening of herbicides. P-PII nucleotide sequences have additional uses as probes for isolating additional genomic clones having the promoters of P-PII gene. P-PII promoters are light- and/or sucrose-inducible and may be advantageously used in genetic engineering of plants.

2. BACKGROUND OF THE INVENTION

Plants can assimilate soil ammonia or nitrate reduced to ammonia into organic form in leaves or roots. Ammonia assimilation into glutamine and glutamate occurs primarily in leaf chloroplasts or in root plastids by the combined action of chloroplast glutamine synthetase (GS2; *GLN2* gene) and glutamate synthase (GOGAT) (Miflin, B.J. & Lea, P.J., 1977, Ann. Rev. Plant Physiol. 28:299-329). As the assimilation of inorganic nitrogen into organic form requires carbon skeletons, reducing equivalents, and ATP, light serves to coordinate nitrogen assimilation with photosynthesis. Genes involved in plant nitrogen assimilation are induced directly by light (via phytochrome), as well as indirectly by metabolic changes in photosynthate. For example, it has been shown that sucrose supplementation to plant growth media can at least partially induce the expression of mRNA for *GLN2* or nitrate reductase (*NR*) in the absence of light (Cheng et al.,1992, Proc. Natl. Acad. Sci. USA. 89:1861-1864; Faure et al., 1994, Plant J. 5:481-491). Conversely, sucrose can repress the expression of asparagine synthetase (*ASN1*) (Lam et al., 1994, Plant Physiol. 106:1347-1357). More recently, it has been shown that the effects of sucrose on gene expression can be reversed by the addition of an organic nitrogen source both for nitrate reductase (*NR*) (Vincentz et al., 1993, Plant J. 3:315-324) and for *ASN1* (Lam et al., 1994, Plant Physiol. 106:1347-1357). These findings indicate that plants are able to sense levels of carbon and organic nitrogen, and in turn modulate the expression of genes involved in nitrogen assimilation.

Bacteria can also assimilate ammonia into glutamate or glutamine. Plants' ability to sense changes in the levels of carbon and nitrogen metabolites is reminiscent of a nitrogen regulatory system (Ntr) in bacteria in which a protein called PII, encoded by the *glnB* gene, can regulate